Manual of Diagnostic Tests for Aquatic Diseases:

Comment to the proposed addition of RT-PCR diagnostic test for spring viremia of carp (SVC)

The United States supports the addition of the RT-PCR test described by Stone et. al (2003) as one of the confirmatory tests for SVC in suspect tissue cultures. However, we have some comments on the proposed protocol:

The proposed protocol describes the methods to differentiate SVCV from antigenically related viruses.

1) It assumes that a viral isolation has been made in cell culture. The proposed RT-PCR protocol described is not concerned with sensitivity or specificity as the primer sets will amplify SVCV, pike fry rhabdovirus, and other antigenically related viruses. Identification/differentiation of the isolated virus occurs by computer evaluation of the cDNA sequence.

2) Perhaps a statement and/or disclaimer should be included in the protocol concerning any specific reference to a trademark or proprietary product does not imply its approval to the exclusion of other products that may be suitable. The proposed RT-PCR protocol should not eliminate the possibility of substituting alternative RNA extraction methods or polymerases. The protocol makes specific reference to Trizol which is a commercial laboratory grade phenol based product. Other commercial and in-house extraction methods will also produce high quality RNA. These products or methods would eliminate the need to handle chemical hazardous waste for laboratories where this may be a problem. There are alternative enzymes (commercial products) that could be used to produce a cDNA for sequencing, For example the polymerase rTth can be used for both reverse transcription and DNA amplification.

3) The RT-PCR reaction conditions described in paragraph three of the proposed protocol differs from the reaction conditions described in Stone *et. al* (2003).

4) There is a typographical error in line six of paragraph three of the proposed protocol. Specifically, the line reads "....each of the SVCV ~~R4~~ and SVCV F1 primers." This should read "....each of the SVCV **R2** and SVCV F1 primers." If primer R4 is actually used in the non-nested PCR a 606 base pair cDNA would be produced.

5) The proposed protocol does not state if the cDNA is sequenced directly or if the product cloned and sequenced.

6) In Stone *et. al* (2003) it indicates that a 550 nucleotide region is used for the phylogenetic analysis? The non-nested PCR generates a 714 bp cDNA product, while the semi-nested PCR generates a 606 bp cDNA product. We suggest listing the relative nucleotide numbers of U18101 that are used for the phylogenetic analysis.

7) Because the proposed protocol amplifies both SVC and Pike Fry Rhabdovirus, the proposed protocol requires virus determination based on sequence homology. The proposed protocol should specify that the isolated virus have some specific level of homology with SVCV subtypes.

8) Adoption of this proposed protocol into the OIE Manual should not prevent the acceptance of future RT-PCR protocols which specifically amplify SVCV and not Pike Fry Rhabdovirus and other antigenically related virus. In fact, development of a more specific RT-PCR should be encouraged.